British Journal of Pharmacology (1996) 117, 764-770

Comparative effects of PACAP and VIP on pancreatic endocrine secretions and vascular resistance in rat

*,[1],†G. Bertrand, *,†R. Puech, *Y. Maisonnasse, *J. Bockaert & †M.M. Loubatières-Mariani

*Centre CNRS-INSERM de Pharmacologie Endocrinologie, UPR 9023, Rue de la Cardonille, 34094 Montepellier Cedex 5, France and †Faculté de Médecine, Laboratoire de Pharmacologie, Institut de Biologie, Boulevard Henri IV, 34060 Montpellier Cedex, France

1 The effects of pituitary adenylate cyclase-activating polypeptide (PACAP), vasoactive intestinal peptide (VIP) and secretin on pancreatic endocrine secretions and vascular resistance were investigated and compared in the isolated perfused pancreas of the rat. The PACAP/VIP receptor types involved have been characterized.

2 On insulin secretion, in the range 10^{-11} to 10^{-8} M, PACAP and VIP elicited a concentration-dependent biphasic response from pancreas perfused with 8.3 mM glucose; the peptides were equipotent. In contrast, secretin was ineffective in the range 10^{-11} to 10^{-9} M; at 10^{-8} and 10^{-7} M, it induced only low and transient insulin responses. On the other hand, the peptides did not modify the basal insulin release in the presence of a non stimulating glucose concentration (2.8 mM).

3 On glucagon secretion, PACAP and VIP (10^{-11} to 10^{-8} M) but also secretin (10^{-9} to 10^{-7} M) caused a concentration-dependent peak shaped response from pancreas perfused with 2.8 mM glucose; PACAP and VIP were equipotent and 20 times more potent than secretin. On the other hand, the peptides did not affect the glucagon release in the presence of 8.3 mM glucose.

4 On pancreatic vessels, in the range 10^{-11} to 10^{-9} M, the three peptides were equipotent in inducing a concentration-dependent sustained increase in pancreatic flow rate. On the other hand, at the high concentration of 10^{-7} M PACAP but not VIP provoked a transient decrease of flow rate.

5 This study provides evidence for PACAP/VIP type II receptors mediating insulin and glucagon secretion as well as vasodilatation in rat pancreas. In addition, the different efficacies of secretin suggest that these effects are mediated by different PACAP/VIP type II receptor subtypes.

Keywords: Pituitary adenylate cyclase-activating polypeptide (PACAP); vasoactive intestinal peptide (VIP); secretin; insulin secretion; glucagon secretion; vessel; rat pancreas

Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide initially from ovine hypothalamus on the basis of its ability to stimulate adenylate cyclase in rat pituitary cells (Miyata *et al.*, 1989). Two biological active forms of the peptide have been identified, one major with 38 amino acids (PACAP-38) and the other with 27 residues (PACAP-27), sharing the same N-terminal 27 amino acids (Miyata *et al.*, 1990). PACAP belongs to the secretin/glucagon/vasoactive intestinal peptide (VIP) family of peptides and shares a high degree of amino acid homology (68%) with VIP (Arimura, 1992). Recent studies have demonstrated the existence of at least two types of high-affinity receptors for PACAP on the basis of their relative affinities for PACAP and VIP, denominated type I and type II (Arimura, 1992; Christophe, 1993; Rawlings, 1994). Type I receptors are PACAP-preferring receptors whereas Type II receptors recognize PACAP and VIP with a similar affinity and appear to be identical with VIP receptors. The Type II receptors (or VIP receptors) have been subdivided into VIP_1 and VIP_2; one pharmacological criterion to distinguish between the two subtypes is the ability of secretin to activate the VIP_1 but not the VIP_2 receptors (see Harmar & Lutz, 1994).

PACAP and VIP are widely distributed, occurring in the central nervous system and peripheral tissues such as pituitary, adrenal, lung, testis, gastrointestinal tract and pancreas where they exert diverse biological effects (Christophe, 1993; Arimura & Shioda, 1995). In the pancreas, PACAP- and VIP-like immunoreactivity has been observed in nerve fibres innervating islets and blood vessels (Bishop *et al.*, 1980; Fridolf *et al.*, 1992; Köves *et al.*, 1993; Yada *et al.*, 1994), suggesting that these peptides may play a physiological role in the control of endocrine secretions and vascular tone. VIP (Schebalin *et al.*, 1977; Szecowka *et al.*, 1980) and more recently PACAP (Kawai *et al.*, 1991; Fridolf *et al.*, 1992; Yokota *et al.*, 1993) have been reported to stimulate insulin and glucagon secretions. However, the PACAP/VIP receptor types involved in these effects have not been elucidated. Indeed, no study has compared the effect of these peptides on glucagon secretion; on the other hand, for insulin secretion it has been reported that PACAP was more potent than VIP suggesting an action at a PACAP receptor type I (Yada *et al.*, 1994) whereas it has been shown that receptors with similar binding properties to the type II are expressed in an insulin-secreting β cell line (Inagaki *et al.*, 1994). In addition, these peptides are known to exhibit a vasolidator effect in various vascular beds (see Christophe, 1993) and VIP has been reported to increase whole pancreatic blood flow in rat (Jansson, 1994).

The present study was designed to compare the effects of PACAP and VIP on pancreatic endocrine secretions and vascular resistance in order to characterize the PACAP/VIP receptor types involved. This work was performed on the isolated perfused pancreas of the rat, a preparation which preserves the anatomical integrity of the islets of Langerhans and pancreatic vessels.

[1]Author for correspondence at: Centre CNRS-INSERM de Pharmacologie Endocrinologie, UPR 9023, Rue de la Cardonille, 34094 Montpellier Cedex 5, France.

Methods

Our experiments were performed with male Wistar rats fed *ad libitum* and weighing 320–350 g. The surgical procedure for the isolated perfused rat pancreas has already been described (Loubatieres *et al.*, 1969; Bertrand *et al.*, 1986). After total isolation from all neighbouring tissues, the pancreas was perfused through its own arterial system and at a constant pressure with a Krebs-Ringer bicarbonate buffer containing 2 g l^{-1} pure bovine serum albumin (fraction V) and glucose 8.3 or 2.8 mM. A mixture of O_2 (95%) and CO_2 (5%) was continuously bubbled through this medium; the pH was about 7.4. The preparation was maintained at 37.5°C. Any change in pancreatic vascular bed resistance induced change in pancreatic effluent output. The pressure (ranging between 40 and 45 cmH_2O) was selected to give a flow rate of 2.5 ml min^{-1} during a 30-min stabilization period.

The first sample was taken after the stabilization period. The test substances were infused for 20 min from time 45 min. The flow rate was measured during 1 min for each sample; an aliquot of 800 µl was immediately frozen in a chilled tube containing 50 µl of a mixture of 32 mM EDTA and 10 000 kiu ml^{-1}aprotinin (Antagosan, Hoechst, Puteaux, France) for glucagon determination and the remainder was frozen for insulin determination.

Insulin was determined by radioimmunoassay using charcoal separation (Herbert *et al.*, 1965), an anti-porcine insulin antibody (ICN, Biochemicals, Orsay, France) and pure rat insulin (Novo, Copenhagen, Denmark) as the reference standard, the biologic activity of which was 14.2 µu ng^{-1}. Glucagon was measured by the method of Unger *et al.* (1970) using the BR 124 glucagon antiserum from the Institut de Biochimie Clinique (Geneva, Switzerland) and porcine glucagon (Novo, Coppenhagen, Denmark) as standard.

For the kinetics of insulin or glucagon output as well as the kinetics of flow rate, the results are expressed as changes in relation to the value at time 45 taken as 100%. Data are expressed as means ± standard error of the mean (s.e.mean).

In order to obtain the concentration-response curves for test substances, we used: (1) for insulin secretion, the increase of mean insulin output over the 20 min of substance infusion calculated as follows: AUC/20 (AUC = area under the curve); (2) for glucagon secretion, the increase in glucagon output rate during the first 5 min of agonist infusion (AUC/5); (3) for flow rate, the mean flow rate over the 20 min of infusion. The values obtained were plotted as a function of the logarithm of peptide concentrations. For each peptide we carried out a linear regression analysis from the values and a comparison of potency was performed by the parallel method (Armitage, 1980).

The data were submitted to analysis of variance followed by Newman-Keuls test for multiple comparisons.

Drugs

VIP and secretion were obtained from Bachem (U.K.). PACAP(1-38) amide was synthesized by the solid phase technique and then purified by reverse phase h.p.l.c. on a semipreparative column (Whatman C_{18}, 10 µm, 22 × 500 mm) according to the procedure of Le Nguyen *et al.* (1987). The purity of the peptide was characterized by analytical h.p.l.c., capillary electrophoresis, mass spectrometry and its composition confirmed by amino acid analysis. The peptides were dissolved in sterile isotonic saline containing 0.2% bovine serum albumin.

Results

Comparative effects of PACAP, VIP and secretin on insulin secretion

In pancreata perfused with the slightly stimulating glucose concentration of 8.3 mM, the insulin output rate in controls was relatively stable and averaged 28.6±4.4 ng min^{-1} at 45 min (the reference value).

The test substances were studied in the concentration range 10^{-11}–10^{-7}M. As shown in Figure 1, in the range 10^{-11} to 10^{-8}M, PACAP induced a biphasic insulin release in a concentration-dependent manner. The increase in mean insulin output rate averaged a maximum of +536±36% with PACAP 10^{-8}M (Figure 3). VIP (10^{-11} to 10^{-8}M) like PACAP, elicited a concentration-dependent biphasic insulin response and a comparable maximal increase of mean insulin output rate: +505±29% at 10^{-8}M (Figures 2a and 3). In contrast, secretin was ineffective in the range 10^{-11}–10^{-9}M and induced only transient weak insulin responses at 10^{-8} and 10^{-7}M (Figures 2b and 3): the insulin output rate peaked within 2 min only at +118±44% and +313±50%, respectively, and then rapidly returned to basal values.

On the other hand, in the presence of a non-stimulating glucose concentration (2.8 mM), these peptides did not affect basal insulin release. The mean insulin output during the 20 min of peptide infusion were 0.8±0.3; 1.2±0.3 and 0.7±0.2 ng with PACAP 10^{-8}M, VIP 10^{-8}M and secretin 10^{-7}M, respectively, versus 1.1±0.4 ng in controls (results not shown).

Comparative effects of peptides on glucagon secretion

When pancreata were perfused with 2.8 mM glucose, the glucagon output rate in controls decreased during the first 50 min and then remained relatively stable (Figure 4).

In the range 10^{-11} to 10^{-8}M, PACAP induced an immediate and transient (5–10 min) glucagon response in a concentration-dependent manner (Figure 4). VIP (10^{-11} to 10^{-8}M) and also secretin (10^{-9} to 10^{-7}M) provoked a concentration-dependent glucagon response with a sharp peak (Figure 5). As shown in Figure 6, the three peptides elicited comparable maximal increase in the mean glucagon output rate: +192±22, +202±29 and +196±16% with PACAP 10^{-8}M,

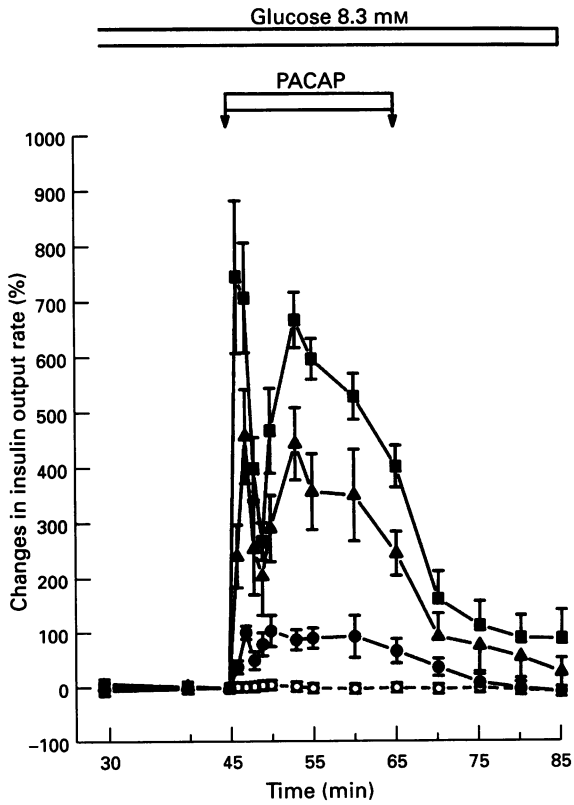
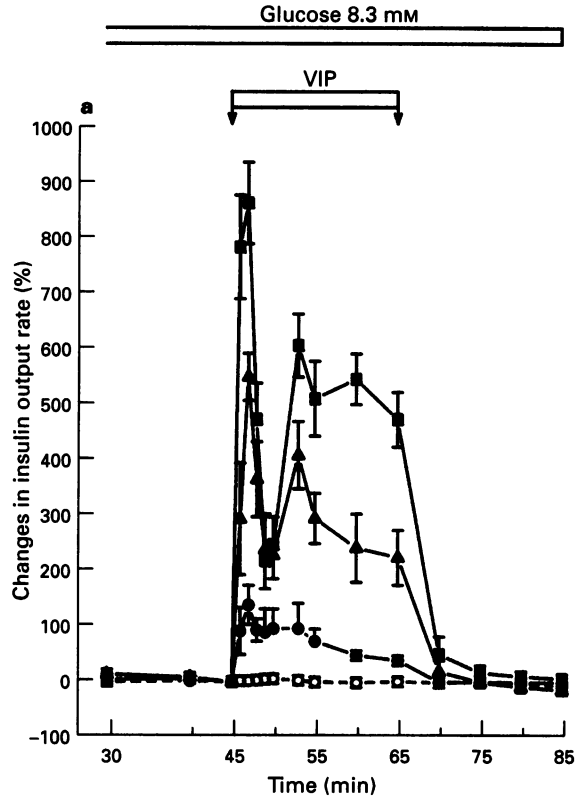


Figure 1 Effects of PACAP on insulin secretion from the rat isolated pancreas perfused with 8.3 mM glucose: (●) 10^{-11}M (n=6); (▲) 10^{-9}M (n=5); (■) 10^{-8}M (n=7); controls (○) (n=12). Each point represents the mean with s.e.mean.



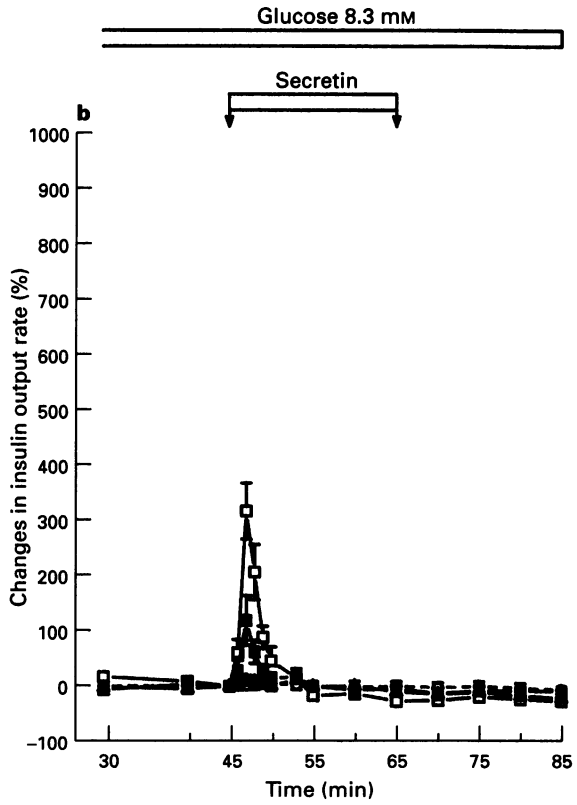


Figure 2 Effects of VIP and secretin on insulin secretion from the rat isolated pancreas perfused with 8.3 mM glucose: (a) VIP, (●) 10^{-11}M ($n=4$); (▲) 10^{-9}M ($n=5$); (■) 10^{-8}M ($n=6$); (b) secretin, (▲) 10^{-9}M ($n=5$); (■) 10^{-8}M ($n=5$); (□) 10^{-7}M ($n=6$). Each point represents the mean with s.e.mean.

VIP 10^{-8}M and secretin 10^{-7}M, respectively. PACAP and VIP were equipotent and were about 20 fold more potent than secretin with [5-45] for 95% confidence limits.

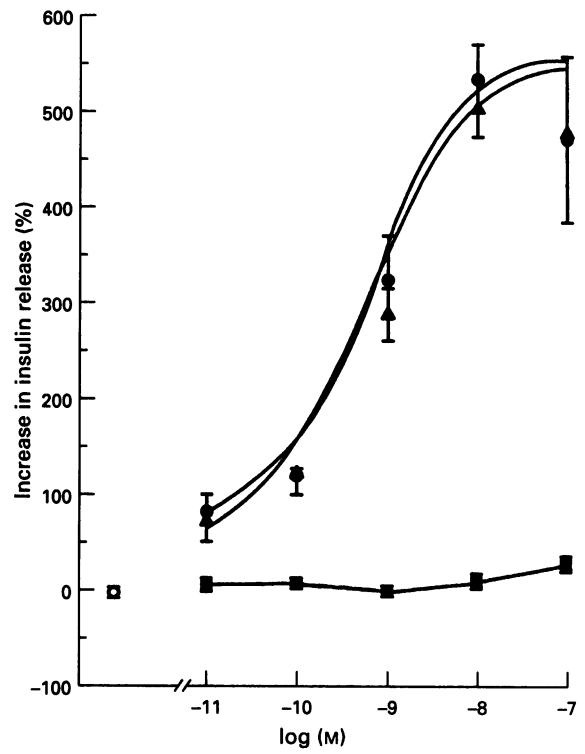


Figure 3 Concentration-response curves for the effects of PACAP (●), VIP (▲) and secretin (■) on insulin secretion from the rat isolated pancreas. Each point represents the mean with s.e.mean of 4–12 experiments.

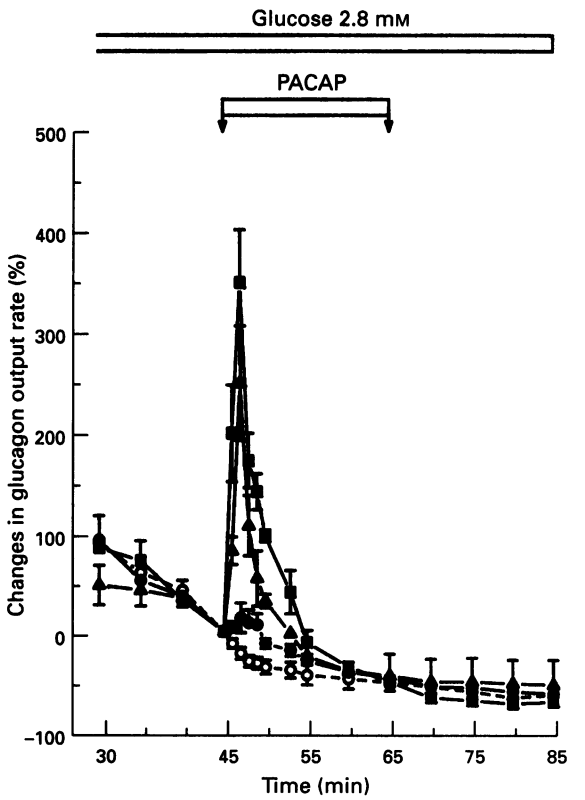


Figure 4 Effects of PACAP on glucagon secretion from the rat isolated pancreas perfused with 2.8 mM glucose: (●) 10^{-11}M ($n=5$); (▲) 10^{-9}M ($n=4$); (■) 10^{-8} ($n=5$); controls (○) ($n=5$). Each point represents the mean with s.e.mean.

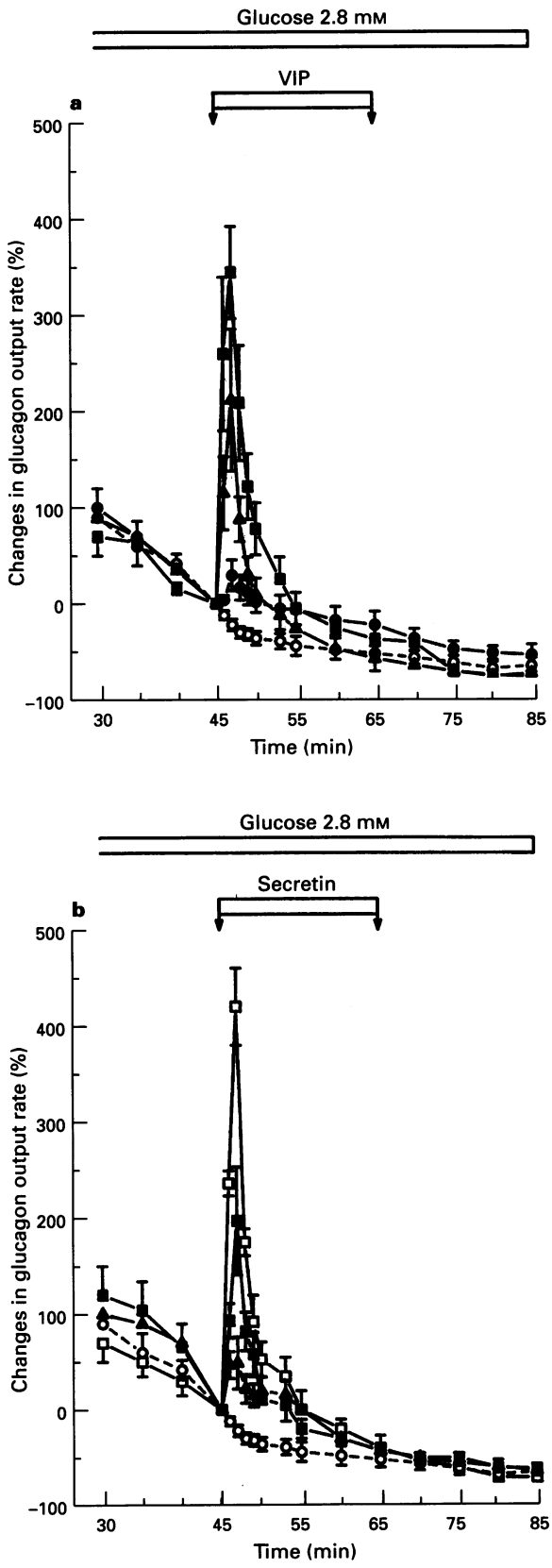


Figure 5 Effects of VIP and secretin on glucagon secretion from the rat isolated pancreas perfused with 2.8 mM glucose: (a) VIP, (●) 10^{-11}M ($n=5$); (▲) 10^{-9}M ($n=6$); (■) 10^{-8}M ($n=6$); (b) secretin, (▲) 10^{-9}M ($n=5$); (■) 10^{-8}M ($n=4$); (□) 10^{-7}M ($n=5$). Each point represents the mean with s.e.mean.

On the other hand, in the presence of 8.3 mM glucose, the peptides did not significantly affect basal glucagon release. The mean glucagon output during the 5 min of peptide infusion

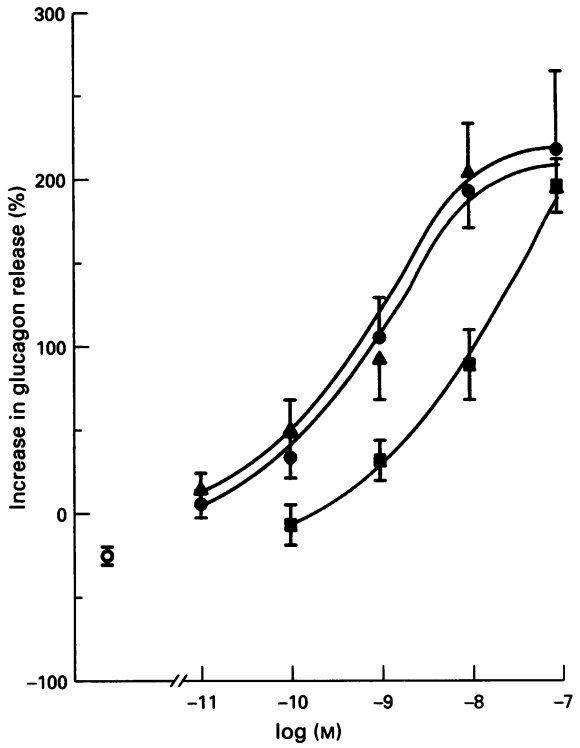


Figure 6 Concentration-response curves for the effects of PACAP (●), VIP (▲) and secretin (■) on glucagon secretion from rat isolated pancreas. Each point represents the mean with s.e.mean of 4–6 experiments.

were 285±35; 302±21 and 383±46 pg with PACAP 10^{-8}M, VIP 10^{-8}M and secretin 10^{-7}M, respectively, versus 315±33 pg in controls (results not shown).

Comparative effects of peptides on pancreatic flow rate

PACAP (10^{-11} to 10^{-9}M) elicited a progressive and sustained increase in the pancreatic flow rate in a concentration-dependent manner (Figure 7). Over the same range of concentrations, both VIP and secretin were effective on the vascular bed (Figure 8). The concentration-response curves of the peptides on flow rate are shown in Figure 9. The three peptides exhibited similar potency and efficacy; they were concentration-dependently effective in the range 10^{-11} to 10^{-9}M and induced similar maximal increases in the mean pancreatic flow rate of about 12%.

In contrast, at the high concentration of 10^{-7}M, PACAP induced an immediate and transient decrease in pancreatic flow rate: the maximum decrease was reached at the second minute (−37±5%) and the return to basal value occurred within 10 min (Figure 10). At this concentration, neither VIP nor secretin elicited a decrease in pancreatic flow rate. In addition VIP, even at the high concentration of 10^{-6}M, elicited a vasodilator effect (results not shown): the mean flow rate during the 10 min infusion was +14±3% versus −17±3% with PACAP 10^{-7}M.

Discussion

The present study shows that PACAP and VIP are equipotent in inducing insulin and glucagon secretion as well as vasodilatation, thus providing evidence for the involvement of PACAP/VIP receptors of type II in the three effects. In addition, the different behaviour of secretin suggests the involvement of PACAP/VIP type II receptors of different subtypes.

PACAP can interact with at least two types of receptors: type I receptors highly specific for PACAP and type II re-

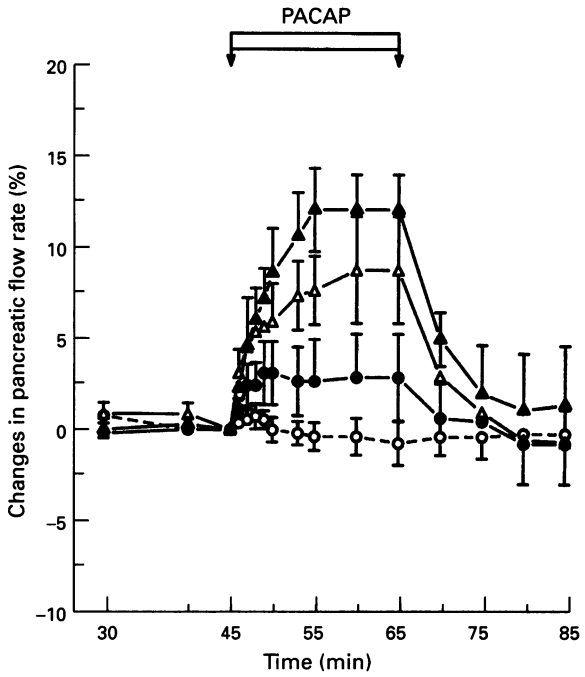
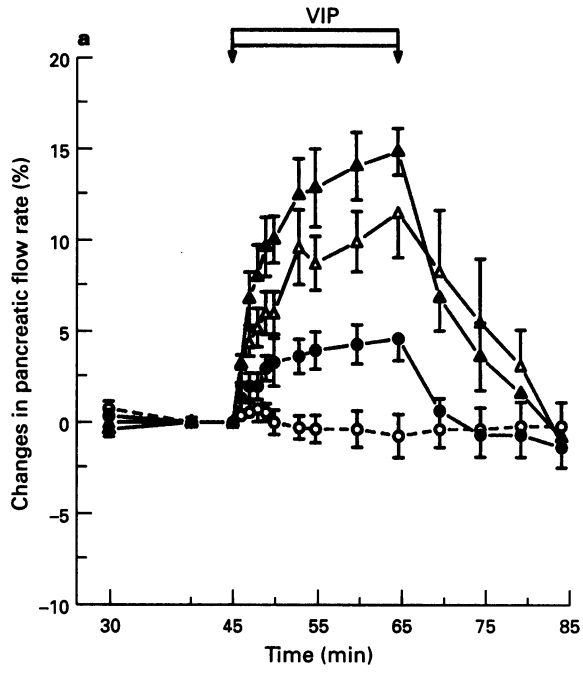


Figure 7 Effects of PACAP on vascular flow rate from the isolated perfused pancreas of the rat: (●) 10^{-11}M ($n=9$); (△) 10^{-10}M ($n=7$); (▲) 10^{-9}M ($n=7$), controls (○)($n=11$). Each point represents the mean with s.e.mean.



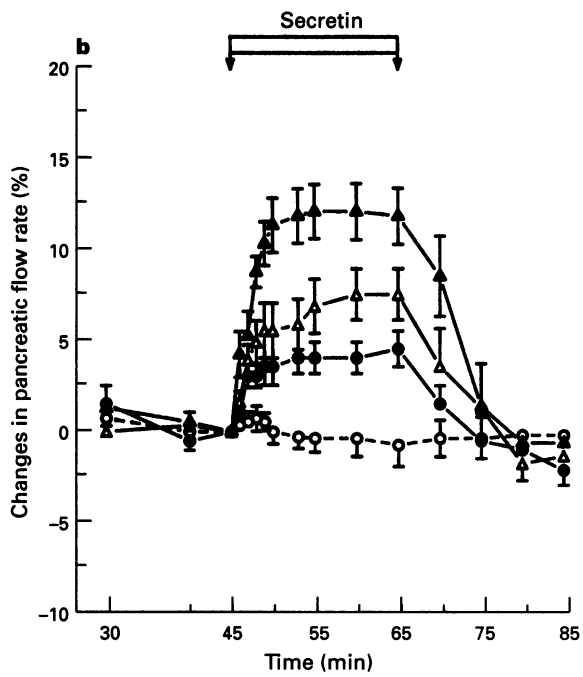


Figure 8 Effects of VIP and secretin on vascular flow rate from the isolated perfused pancreas of the rat: (a) VIP, (●) 10^{-11}M ($n=6$); (△) 10^{-10}M ($n=5$); (▲) 10^{-9}M ($n=5$) (b) secretin, (●) 10^{-11}M ($n=4$); (△) 10^{-10}M ($n=6$); (▲) 10^{-9}M ($n=8$). Each point represents the mean with s.e.mean.

ceptors which show a similar affinity for PACAP and VIP (Arimura, 1992; Christophe, 1993). The type II receptors which appear to be classic VIP receptors, have been subdivided into VIP_1 and VIP_2 on the basis of cloning and functional expression studies. One major pharmacological difference between the two subtypes is the ability of secretin to activate the VIP_1 receptor but not the VIP_2 receptor (Ishihara *et al.*, 1992; Lutz *et al.*, 1993; Usdin *et al.*, 1994).

On insulin secretion, PACAP and VIP induced a concentration-dependent biphasic response in the range 10^{-11} to 10^{-8}M. The peptides exhibited comparable potency and efficacy which is consistent with the involvement of PACAP/VIP receptors of type II (or VIP receptor) mediating insulin secretion. Our results conflict with those of a recent study which reported that PACAP (27 or 38) stimulated insulin release from rat pancreatic islets at sub-picomolar concentrations with a maximum at 10^{-13}M and that the peptide was 4 log units more potent than VIP in increasing cytosolic Ca^{2+} concentration in B cells; the authors therefore concluded that the effects of PACAP are mediated by a PACAP selective type I receptor (Yada *et al.*, 1994). In contrast, it has been reported that PACAP-27 was concentration-dependently effective in the range 10^{-10} to 10^{-8}M and appeared to be only about ten fold more potent than VIP on insulin secretion from rat isolated pancreas perfused with 5.5 mM glucose (Kawai *et al.*, 1991). These apparent discrepancies may be explained by the different experimental conditions used. Furthermore, in our study, the failure for secretin to elicit, as did PACAP and VIP, a biphasic insulin secretion suggests a PACAP/VIP type II receptor of VIP_2 subtype mediating insulin release. Our data support a recent study reporting the cloning from an insulin-secreting pancreatic cell of a PACAP receptor type 3 which is homologous with the VIP_2 subtype (Inagaki *et al.*, 1994). In addition, Usdin *et al.* (1994) have reported that the VIP_2 receptor mRNA has been found in pancreatic islets which are mainly composed of insulin secreting β cells (about 80%).

PACAP and VIP (10^{-11} to 10^{-8}M) induced a concentration-dependent peak-shaped response on glucagon secretion with comparable potency and efficacy. Various studies have reported that glucagon secretion was stimulated by VIP (Schebalin *et al.*, 1977; Szecowka *et al.*, 1980) and more recently by PACAP (Fridolf *et al.*, 1992; Yokota *et al.*, 1993). However, this is the first study which compares the effects of PACAP and VIP under the same experimental conditions. In addition, in contrast to what is observed on insulin secretion, secretin is effective although 20 fold less potent; the apparent rank order of agonist potency was PACAP = VIP > secretin. Thus, our data are consistent with PACAP/VIP type II receptors mediating glucagon secretion and suggest the involvement of receptors of the VIP_1 subtype rather than the VIP_2 subtype.

The insulin and glucagon secretory effects of PACAP and VIP were dependent on the glucose concentration: whereas they stimulated insulin secretion in the pancreas perfused with a moderate stimulatory glucose concentration (8.3 mM), they

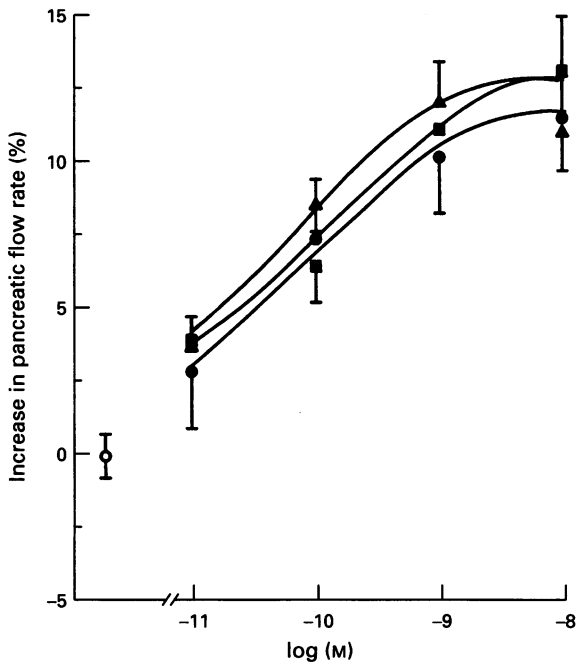


Figure 9 Concentration-response curves for the effects of PACAP (●), VIP (▲) and secretin (■) on pancreatic flow rate. Each point represents the mean with s.e.mean of 4-11 experiments.

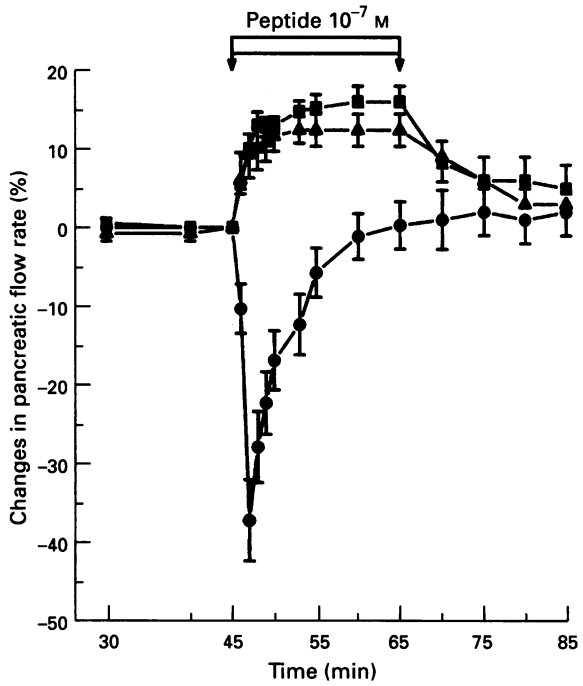


Figure 10 Comparative effects of the peptides at 10^{-7}M on pancreatic flow rate: (●) PACAP ($n=7$); (▲) VIP ($n=5$) and (■) secretin ($n=8$).

were ineffective on basal insulin secretion in the presence of a low glucose concentration (2.8 mM). These peptides are not initiators but potentiators of glucose-induced insulin release. In contrast, they stimulated glucagon secretion at low glucose concentration but not at 8.3 mM glucose. Thus, pancreatic glucagon and insulin cells have different sensitivities to peptides according to the glucose concentration. As insulin and glucagon are respectively hypo- and hyperglycaemic hormones, PACAP and VIP could play a role in the regulation of glycaemia.

On the pancreatic flow rate PACAP and VIP induced a vasodilator effect in the range 10^{-11} to 10^{-9}M and were equipotent. It is well known that PACAP and VIP vasodilate various vascular beds but differences in their potency have been reported. Thus in rat, PACAP exhibited a similar potency to VIP in decreasing systemic arterial pressure (Nandha *et al.*, 1991; Absood *et al.*, 1992) and relaxing tail or mesenteric arteries (Absood *et al.*, 1992; Huang *et al.*, 1993). In contrast, PACAP has been reported to be 100 fold more potent than VIP in relaxing rabbit aorta (Warren *et al.*, 1991). These discrepancies may be explained by differences in species and/or in vessels studied. In our study, the fact that PACAP and VIP exhibited comparable potency provides evidence for PACAP/VIP type II receptors mediating vasodilatation in rat pancreas. In addition, the ability of secretin to exhibit such an effect suggests the involvement of VIP_1 receptors rather than VIP_2 receptors. Our data are supported by a recent study showing by *in situ* hybridization that VIP_1 receptor mRNA is clearly associated with blood vessels in rat pancreas (Usdin *et al.*, 1994). It has been shown that VIP increased whole pancreas blood flow without affecting islet blood flow in rat *in vivo* and thus, there is no correlation between increase in islet blood flow and hormone secretions (Jansson, 1994). On the other hand, it must be noted that, at the high concentration of 10^{-7}M, the vasodilator effect of PACAP was reversed to a clear transient decrease in pancreatic flow rate. This vasoconstrictor effect was not recorded with VIP even at the high concentration of 10^{-6}M. In cats, PACAP has been reported to cause, at high doses, biphasic changes (an initial decrease followed by an increase) in both arterial pressure and systemic vascular resistance, whereas VIP produced only decreases. In these *in vivo* studies, the pressor responses to PACAP were abolished by α-adrenoceptor blockade or by adrenalectomy, suggesting that they are mediated by an α-adrenoceptor mechanism that is dependent on the release of adrenal catecholamines (Minkes *et al.*, 1992; Santiago *et al.*, 1993). Such an effect cannot occur in our *in vitro* study and, although the vasoconstrictor response to PACAP was recorded at a high concentration (10^{-7}M), the failure of VIP to induce this response may suggest the involvement of specific PACAP type I receptors. Further studies are required to confirm this.

In conclusion, PACAP and VIP are equipotent in inducing insulin and glucagon secretions as well as vasodilatation which is indicative of the involvement of PACAP/VIP receptors of type II. In addition, the different efficacies of secretin suggest that these effects are mediated by different PACAP/VIP type II receptor subtypes: insulin secretion appears to be mediated by VIP_2 subtype whereas glucagon secretion and vasodilatation seem to involve VIP_1 subtypes.

We wish to thank J. Boyer and C. Clement for expert technical assistance and V. Montesinos for editorial help.

References

ABSOOD, A., CHEN, D., WANG, Z.Y. & HAKANSON, R. (1992). Vascular effects of pituitary adenylate cyclase activating peptide: a comparison with vasoactive intestinal peptide. *Regulat. Pept.*, **40**, 323–329.

ARIMURA, A. (1992). Pituitary adenylate cyclase activating polypeptide (PACAP): discovery and current status of research. *Regulat. Pept.*, **37**, 287–303.

ARIMURA, A. & SHIODA, S. (1995). Pituitary adenylate cyclase activating polypeptide (PACAP) and its receptors: neuroendocrine and endocrine interaction. *Front Neuroendocrinol.*, **16,** 53–88.

ARMITAGE, P. (1980). Biological assay. In *Statistical Methods in Medical Research.* ed. Armitage P. pp. 442–456. Oxford: Blackwell.

BERTRAND, G. , CHAPAL, J. & LOUBATIERES-MARIANI, M.M. (1986). Potentiating synergism between adenosine diphosphate or triphosphate and acetylcholine on insulin secretion. *Am. J. Physiol.*, **251,** E416–E421.

BISHOP, A.E., POLAK, J.M., GREEN, I.C., BRYANT, M.G. & BLOOM, S.R. (1980). The location of VIP in the pancreas of man and rat. *Diabetologia*, **18,** 73–78.

CHRISTOPHE, J. (1993). Type I receptors for PACAP (a neuropeptide even more important that VIP?). *Biochim. Biophys. Acta.*, **1154,** 183–199.

FRIDOLF, T., SUNDLER, F. & AHREN, B. (1992). Pituitary adenylate cyclase-activating polypeptide (PACAP): occurrence in rodent pancreas and effects on insulin and glucagon secretion in the mouse. *Cell Tissue Res.*, **269,** 275–279.

HARMAR, T. & LUTZ, E.M. (1994). Multiple receptors for PACAP and VIP. *Trends Pharmacol. Sci.*, **15,** 97–99.

HERBERT, V., LAW, K.S., GOTTLIEB, C.W. & BLEICHER, S.J. (1965). Coated charcoal immunoassay of insulin. *J. Clin. Endocrinol.*, **25,** 1375–1384.

HUANG, M., SHIRAHASE, H. & RORSTAD, O.P. (1993). Comparative study of vascular relaxation and receptor binding by PACAP and VIP. *Peptides*, **14,** 755–762.

INAGAKI, N., YOSHIDA, H., MIZUTA, M., MIZUNO, N., FUJIII, Y., GONOI, Y., MIYAZAKI, J.I. & SEINO, S. (1994). Cloning and functional characterization of a third pituitary adenylate cyclase-activating polypeptide receptor subtype expressed in insulin-secreting cells. *Proc. Natl. Acad. Sci. U.S.A.*, **91,** 2679–2683.

ISHIHARA, T., SHIGEMOTO, T., MORI, K., TAKAHASHI, K. & NAGATA, S. (1992). Functional expression and tissue distribution of a novel receptor for vasoactive intestinal polypeptide. *Neuron*, **8,** 811–819.

JANSSON, L. (1994). Vasoactive intestinal polypeptide increases whole pancreatic blood flow but does not affect islet blood flow in the rat. *Acta Diabetol.*, **31,** 103–106.

KAWAI, K., OHSE, C., WATANABE, Y., SUZUKI, S., YAMASHITA, K. & OHASHI, S. (1991). Pituitary adenylate cyclase activating polypeptide stimulates insulin release from the isolated perfused rat pancreas. *Life Sci.*, **50,** 257–261.

KÖVES, K., ARIMURA, A., VIGH, S., SOMOGYVARI-VIGH, A. & MILLER, J. (1993). Immunohistochemical localization of PACAP in the ovine digestive system. *Peptides*, **14,** 449–455.

LE NGUYEN, D., HEITZ, A. & CASTRO, B. (1987). Rapid solid-phase synthesis of the rattine sequence tetradecapeptide using BOP reagent. *J. Chem. Soc. Perkin Trans.*, **I,** 1915–1919.

LOUBATIERES, A.L., MARIANI, M.M., DE MALBOSC, H., RIBES, G. & CHAPAL, J. (1969). Etude expérimentale d'un nouveau sulfamide hypoglycémiant particuliérement actif, le HB 419 ou glibenclamide. I. Action bêtacytotrope et insulino-sécrétrice. *Diabetologia*, **5,** 1–10.

LUTZ, E.M., SHEWARD, W.J., WEST, K.M., MORROW, J.A., FINK, G. & HARMAR, A.J. (1993). The VIP_2 receptor: molecular characterisation of a cDNA encoding a novel receptor for vasoactive intestinal peptide. *FEBS Letts.*, **334,** 3–8.

MINKES, R.K., MCMAHON, T.J., HIGUERA, T.R., MURPHY, W.A., COY, D.H. & KADOWITZ, P.J. (1992). Analysis of systemic and pulmonary vascular responses to PACAP and VIP: role of adrenal catecholamines. *Am. J. Physiol.*, **263,** H1659–H1669.

MIYATA, A., ARIMURA, A., DAHL, R.D., MINAMINO, N., UEHARA, A., JIANG, L., CULLER, M.D. & COY, D.H. (1989). Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. *Biochem. Biophys. Res. Commun.*, **164,** 567–574.

MIYATA, A., JIANG, L., DAHL, R.D., KITADA, C., KUBO, K., FUJINO, M., MINAMINO, N. & ARIMURA, A. (1990). Isolation of a neuropeptide corresponding to the N-terminal 27 residues of the pituitary adenylate cyclase activating polypeptide with 38 residues (PACAP38). *Biochem. Biophys. Res. Commun.*, **170,** 643–648.

NANDHA, K.A., BENITO-ORFILA, M.A., SMITH, D.M., GHATEI, M.A. & BLOOM, S.R. (1991). Action of pituitary adenylate cyclase-activating polypeptide and vasoactive intestinal polypeptide on the rat vascular system: effects on blood pressure and receptor binding. *J. Endocrinol.*, **129,** 69–73.

RAWLINGS, S.R. (1994). PACAP, PACAP receptors, and intracellular signalling. *Mol. Cell. Endocrinol.*, **101,** C5–C9.

SANTIAGO, J.A. & KADOWITZ, P.J. (1993). Analysis of responses to pituitary adenylate cyclase activating polypeptide-38 in the feline hindquarters vascular bed. *Eur. J. Pharmacol.*, **243,** 291–294.

SCHEBALIN, M., SAID, S.I. & MAKHLOUF, G.M. (1977). Stimulation of insulin and glucagon secretion by vasoactive intestinal peptide. *Am. J. Physiol.*, **232,** E197–E200.

SZECOWKA, J., SANDBERG, E. & EFENDIC, S. (1980). The interaction of vasoactive intestinal polypeptide (VIP), glucose and arginine on the secretion of insulin, glucagon and somatostatin in the perfused rat pancreas. *Diabetologia*, **19,** 137–142.

UNGER, R.H., AGUILAR-PARADA, E., MULLER, W. & EISENTRAUT, A.M. (1970). Studies of pancreatic alpha cell function in normal and diabetic subjects. *J. Clin. Invest.*, **49,** 837–848.

USDIN, T.B., BONNER, T.I. & MEZEY, E. (1994). Two receptors for vasoactive intestinal polypeptide with similar specificity and complementary distributions. *Endocrinology*, **135,** 2662–2680.

WARREN, J.B., DONNELLY, L.E., CULLEN, S., ROBERTSON, B.E., GHATEI, M.A., BLOOM, S.T. & MAC-DERMOT, J. (1991). Pituitary adenylate cyclase-activating polypeptide: a novel, long-lasting, endothelium-independent vasorelaxant. *Eur. J. Pharmacol.*, **197,** 131–134.

YADA, T., SAKURADA, M., IHIDA, K., NAKATA, M., MURATA, F., ARIMURA, A. & KIKUCHI, M. (1994). Pituitary adenylate cyclase activating polypeptide is an extraordinary potent intra-pancreatic regulator of insulin secretion from islet β-cells. *J. Biol. Chem.*, **269,** 1290–1293.

YOKOTA, C., KAWAI, K., OHASHI, S., WATANABE, Y., SUZUKI, S. & YAMASHITA, K. (1993). Stimulatory effects of pituitary adenylate cyclase-activating polypeptide (PACAP) on insulin and glucagon release from the isolated perfused rat pancreas. *Acta Endocrinol.*, **129,** 473–479.

(Received July 28, 1995
Accepted October 20, 1995)